Aging & Rehabilitation

An Interdisciplinary Research Seminar Series

Sponsors

Department of Veteran Affairs

- Rehabilitation Outcomes Research Center (RORC)
- Brain Rehabilitation Outcomes Research Center (BRRC)
- Geriatric Research, Education, and Clinical Center (GRECC)
- Support Staff: Nan Musson, Ph.D., BRRC and Sharon Anderson, M.S., GRECC

UF College of Medicine

- Institute on Aging
- Department of Aging and Geriatric Research
- Support Staff: Louise Perras

UF College of Public Health and Health Professions

- Brooks Center for Rehabilitation Studies

UF College of Liberal Arts and Sciences

- Center for Gerontological Studies

UF McKnight Brain Institute

UF College of Nursing

Schedule

- January 9th, 2006 – May 22nd, 2006
- Mondays, 12:00 – 1:00
- Location: UF HPNP Building, Room G101
- Cyber Seminar:
  - VA RORC Conference Room, Commerce Building Downtown
  - VA BRRC Nursing Home Care Unit Conference Room (first floor)
  - UF Brooks Center Conference Room, Jacksonville (904) 306-8977

Themes

- Basic Science (Leeuwenburgh)
- Clinical Science (Beyth)
- Outcomes / Health Policy (Andresen)
- Behavioral and Social Research (Marsiske)
- Cutting Edge / New Research (Aris/Foster)

- Please contact theme chairs to suggest speakers or contact me directly: cleeuwen@aging.ufl.edu

Information on Today's Speaker

- Name: Thomas Wronski, PhD
- Department: Department of Physiological Sciences,

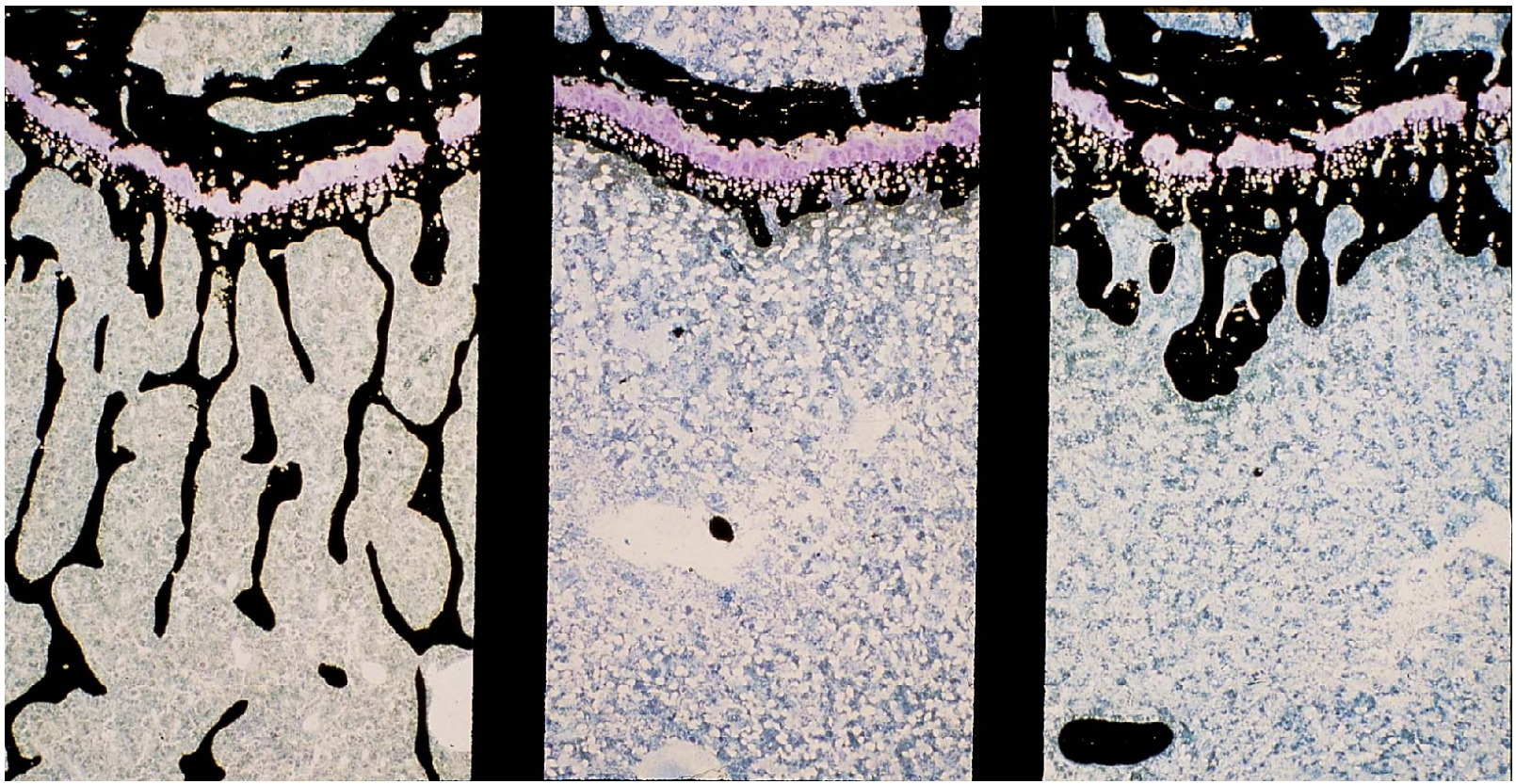
  College of Veterinary Medicine
- Education:
  - B.S. in Biology, 1972, St. Joseph's College, Philadelphia PA
  - Ph.D. in Anatomy 1979, University of Utah, Salt Lake City UT
- Research Interests: Preclinical testing of potential treatments for postmenopausal osteoporosis.

PRECLINICAL TESTING OF POTENTIAL TREATMENTS FOR SEVERE OSTEOPORISIS

POSTMENOPAUSAL OSTEOPOROSIS

- Nearly 50% of women over 65 years of age will experience bone fractures after only minimal trauma.
- National cost for health care of osteoporotic patients is estimated to be 18 billion annually.
- Most of the FDA-approved treatments for osteoporosis are anti-resorptive agents, which prevent additional bone loss from occurring, but do not restore lost bone to normal levels.

PTH FAILS TO RESTORE LOST CANCELLOUS BONE AT A SEVERELY OSTEOPENIC SKELETAL SITE IN AGED OVX RATS



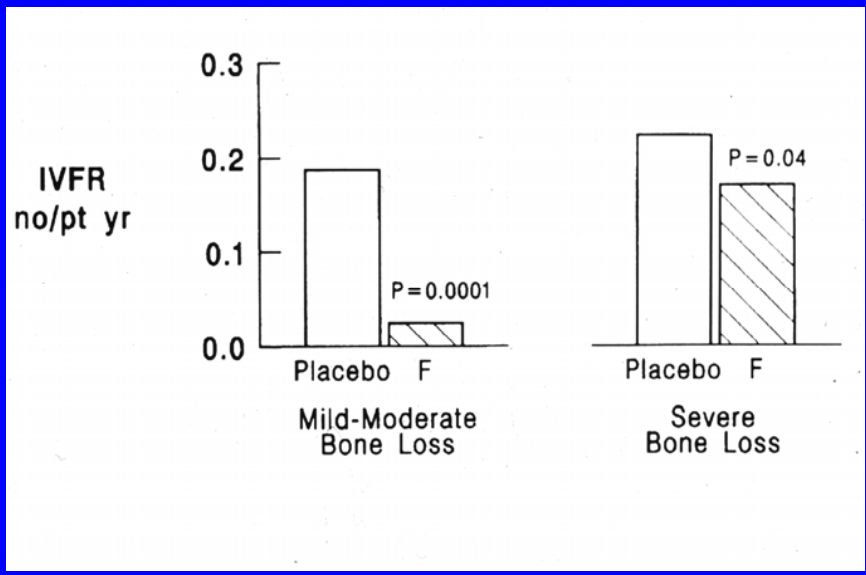
CONTROL OVX OVX+PTH

SEVERITY OF OSTEOPOROSIS LIMITS THE EFFECTIVENESS OF BONE ANABOLIC THERAPY IN HUMANS TOO!

Effect of Slow-Release Sodium Fluoride on Cancellous Bone Histology and Connectivity in Osteoporosis

J. E. ZERWEKH,[1] H. K. HAGLER,[2] K. SAKHAEE,[1] F. GOTTSCHALK,[3] R. D. PETERSON,[1] and C. Y. C. PAK[1]

When patients were divided into those with severe and mild-modest spinal bone loss (based upon initial lumbar bone density) the significant changes in connectivity occurred in patients with mild-moderate bone loss, but not in those with severe bone loss, suggesting that fluoride's effect is in part dependent on the presence of a certain critical amount of bone.



BASIC FIBROBLAST GROWTH FACTOR (bFGF OR FGF-2)

- Stimulates proliferation of osteoblast-like cells and mesenchymal stem cells in vitro
- Induces mesenchymal stem cells to form bone-like nodules in vitro
- Stimulates bone formation and creates new bone spicules within the bone marrow of intact rats

EXPERIMENTAL GROUPS

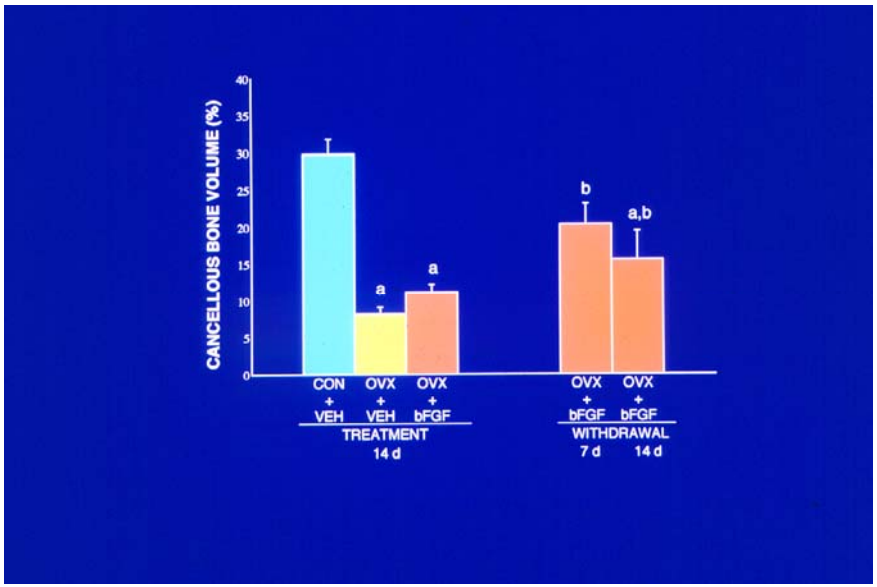
1. Control + vehicle
2. OVX + vehicle
3. OVX + bFGF (treatment)

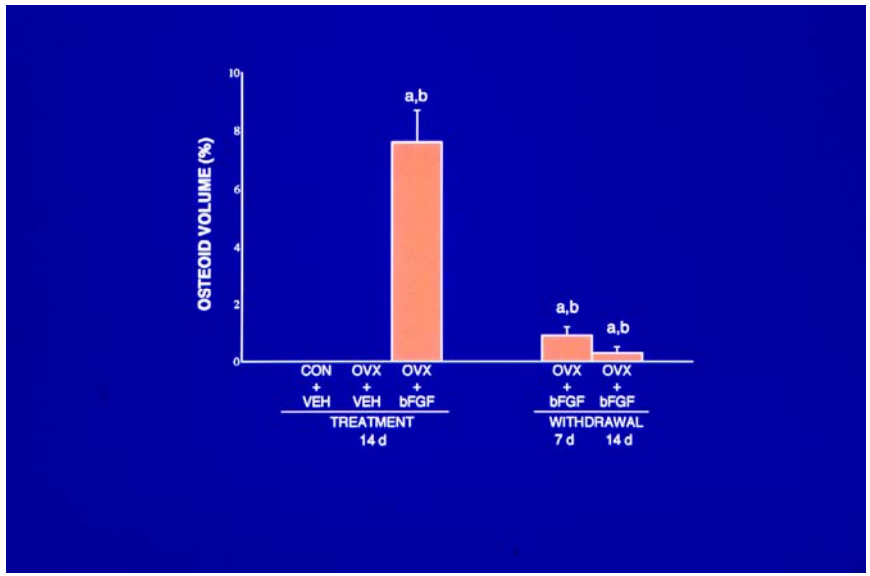
   -daily IV injections of bFGF for 14 days

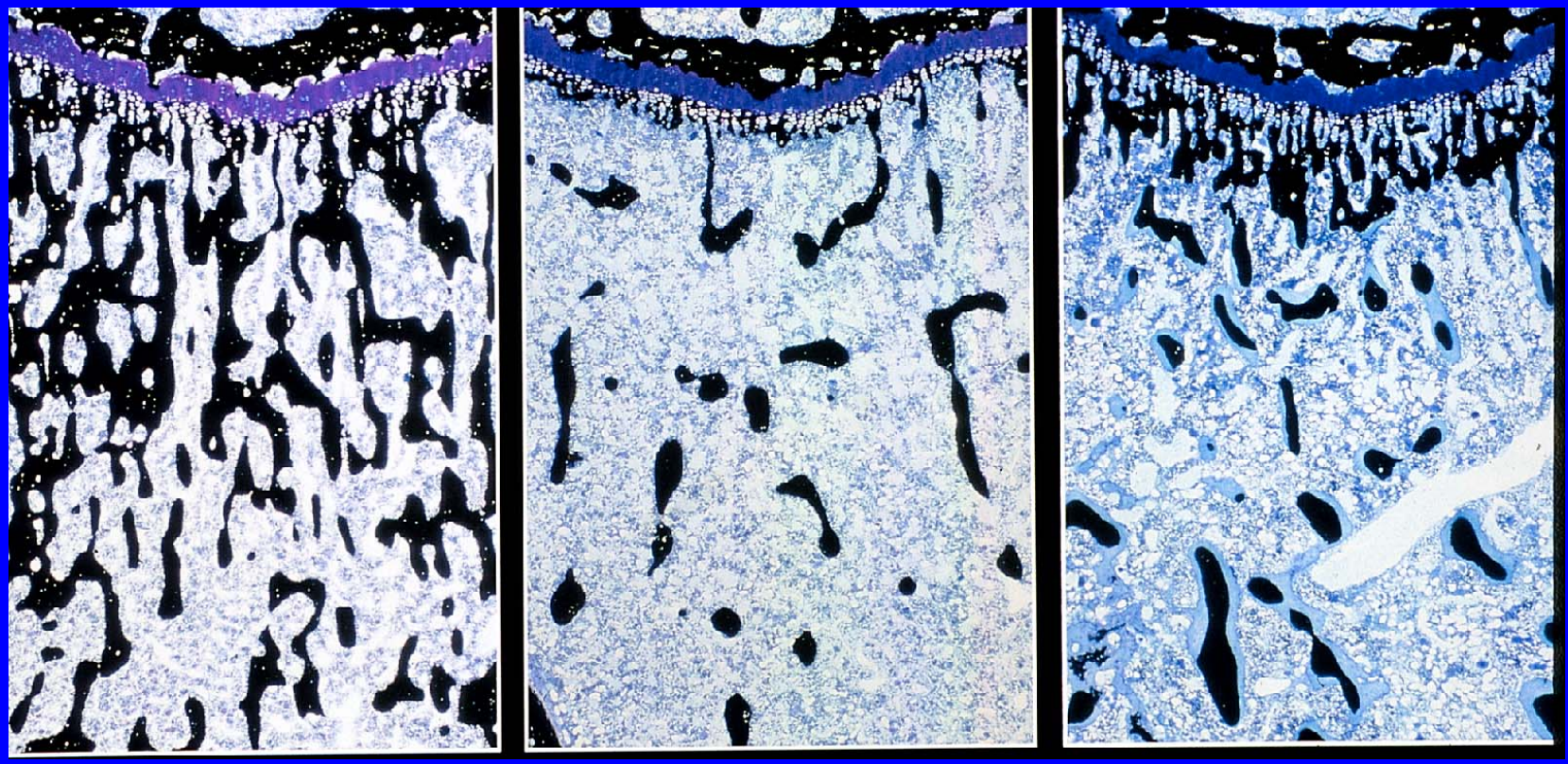
   at a dose of 200 μg/kg
4. OVX + bFGF (withdrawal)

   -sacrificed at 7 or 14 days after withdrawal of bFGF treatment

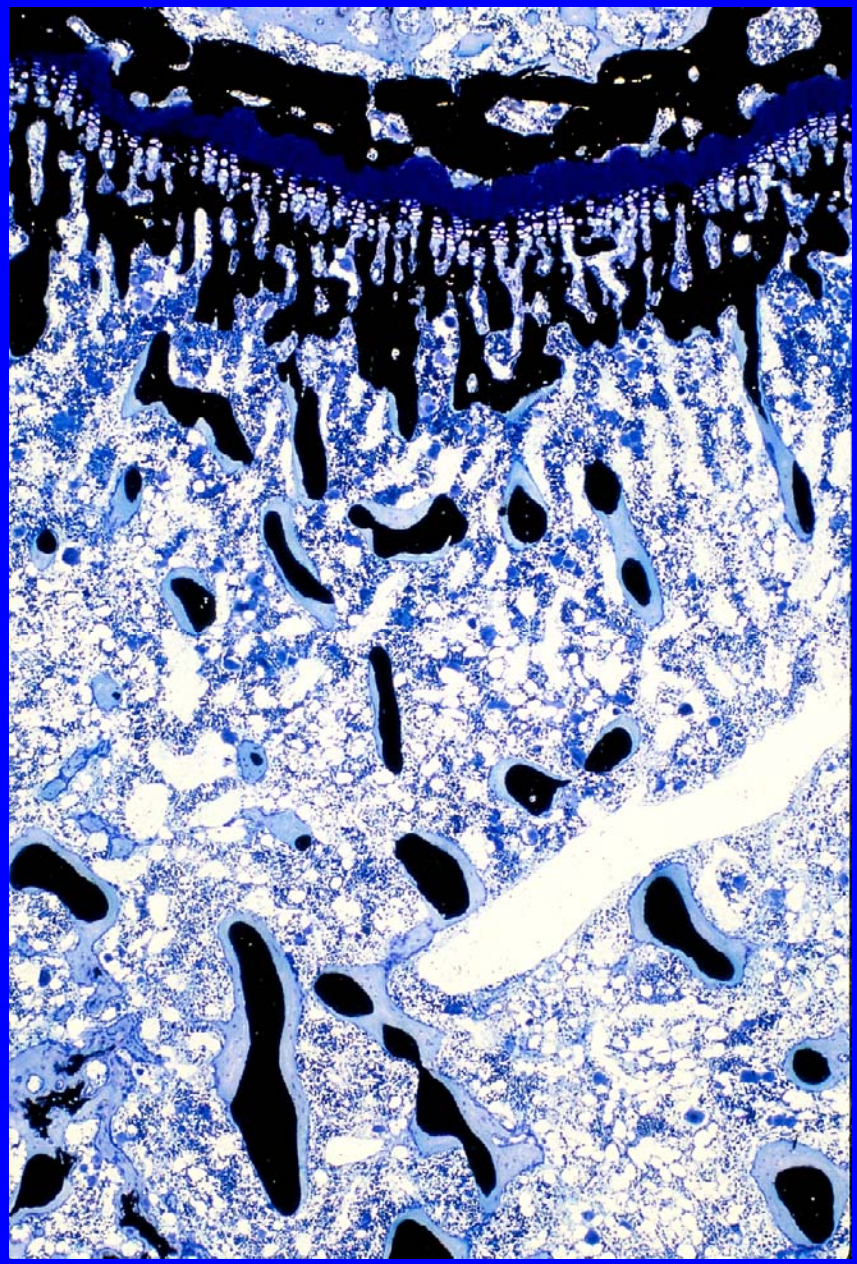
BASIC FGF MARKEDLY INCREASES THE SYNTHESIS OF NEW BONE MATRIX (OSTEOID)

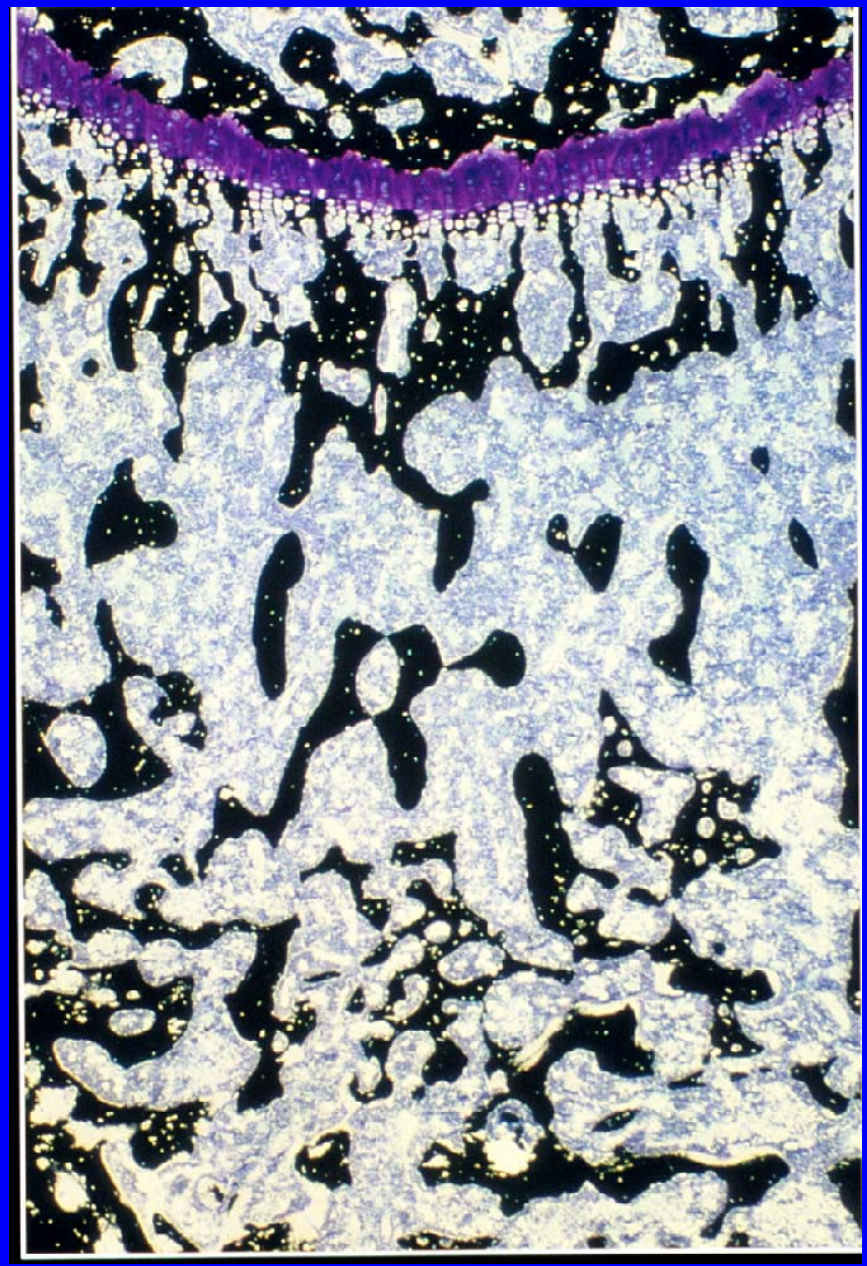




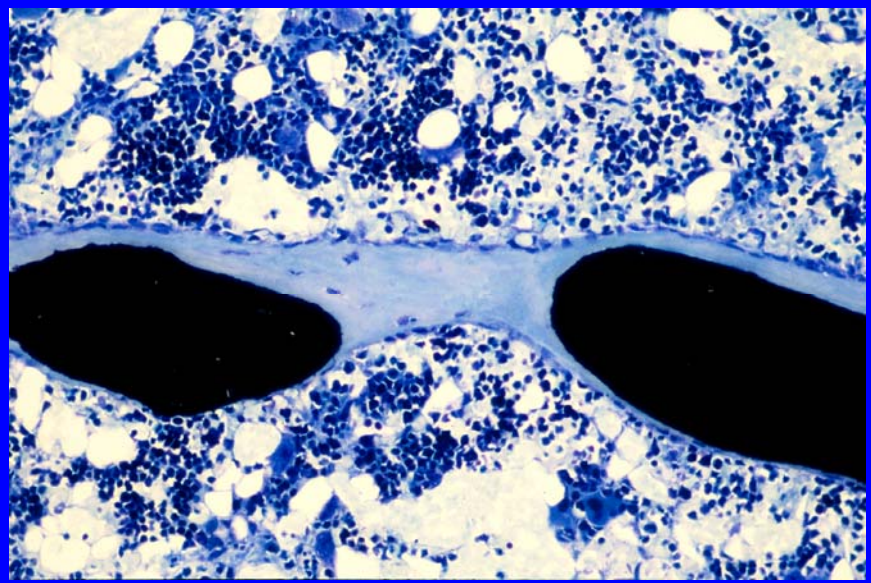


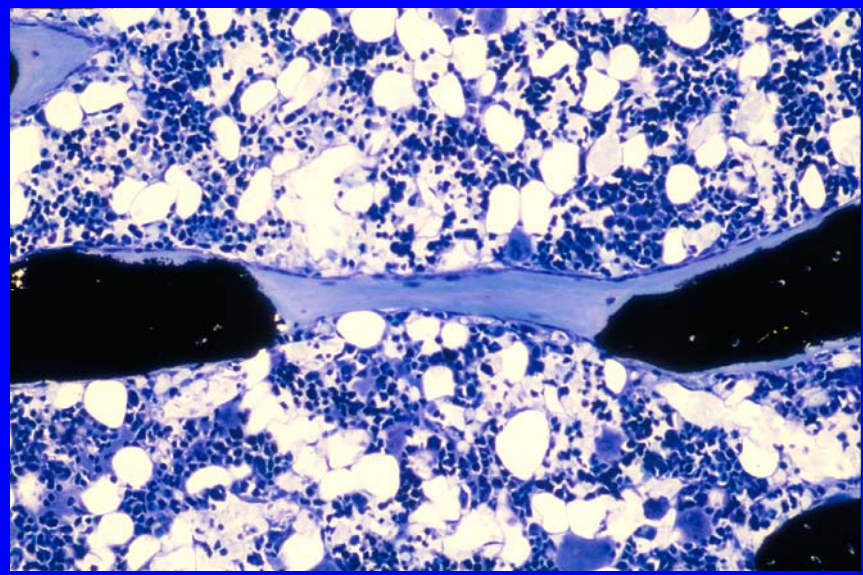
CONTROL+VEH OVX+VEH OVX+bFGF



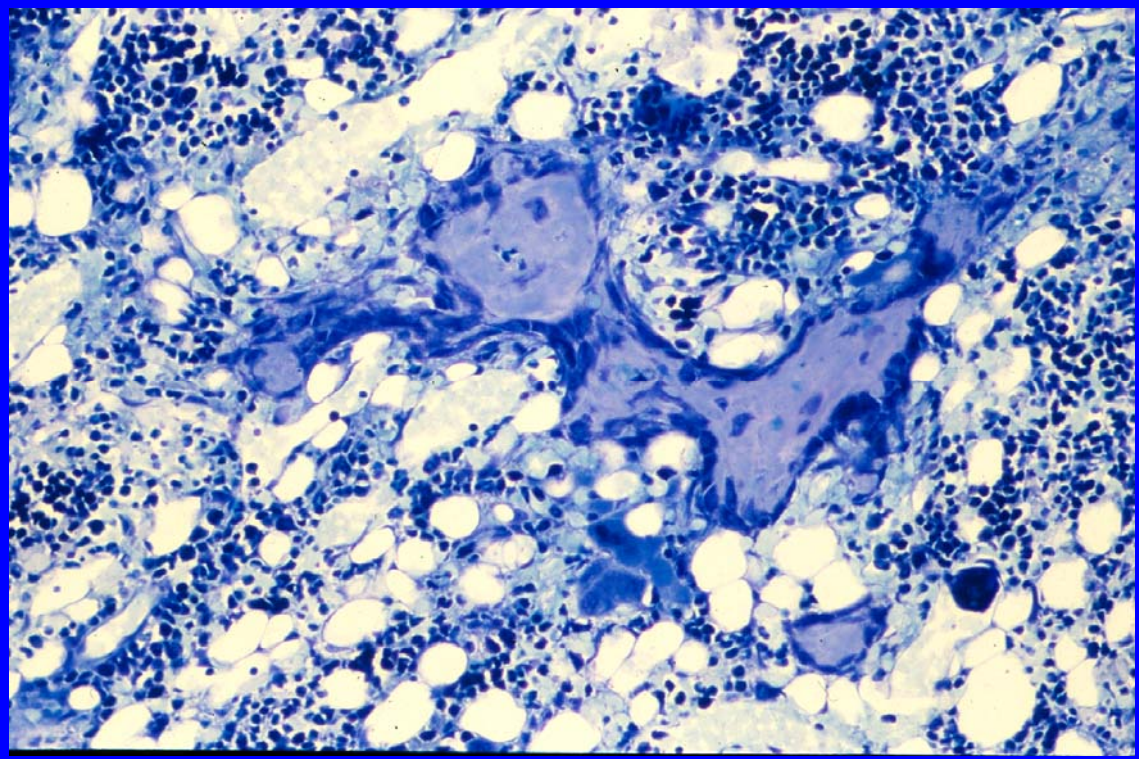


bFGF INDUCES FORMATION OF OSTEOID BRIDGES BETWEEN PRE-EXISTING BONE SPICULES

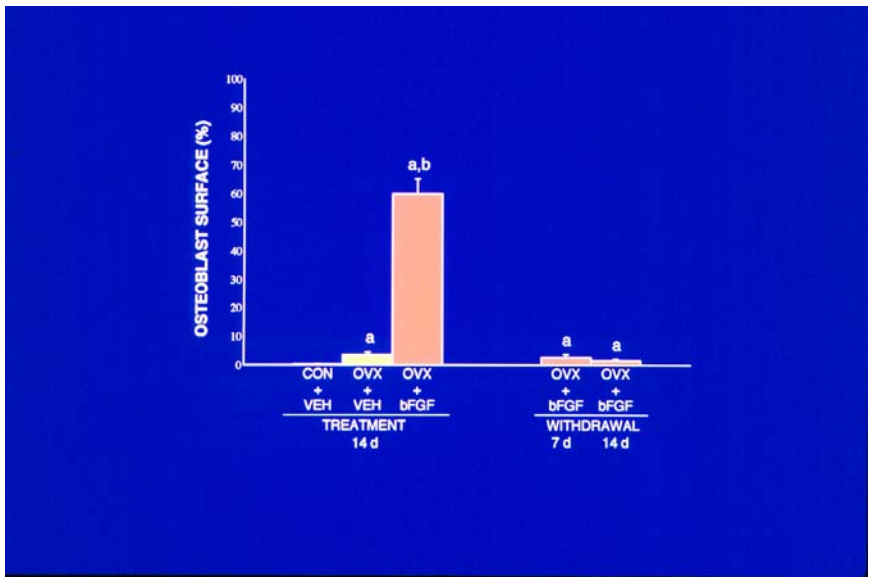


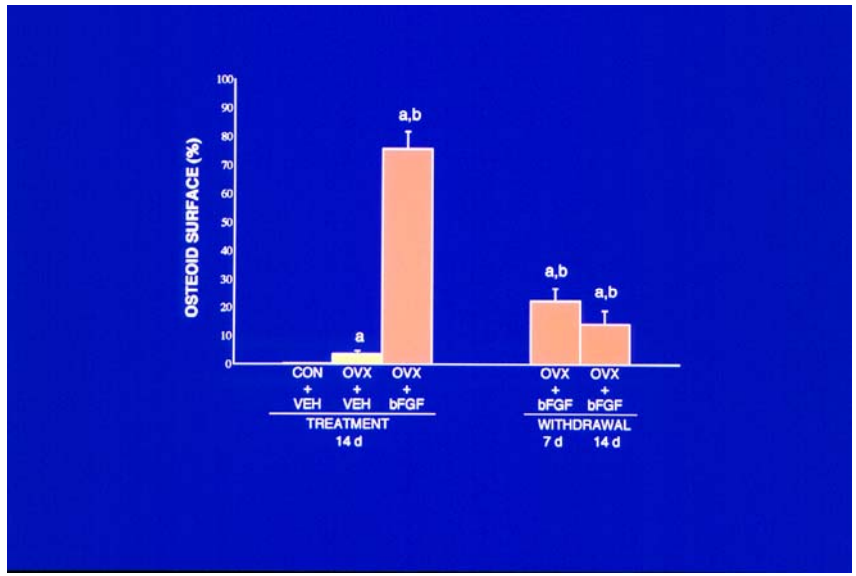


bFGF INDUCES FORMATION OF OSTEOID ISLANDS WITHIN BONE MARROW

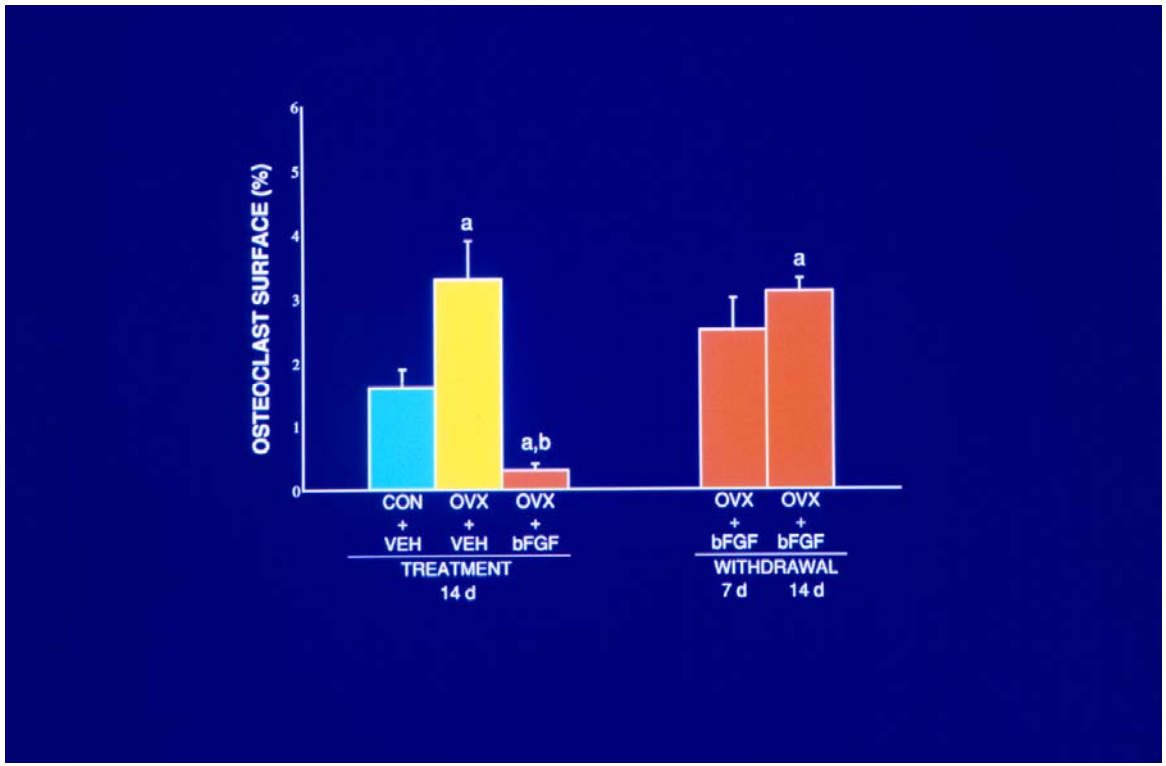


bFGF MARKEDLY INCREASES OSTEOBLAST AND OSTEOID SURFACES





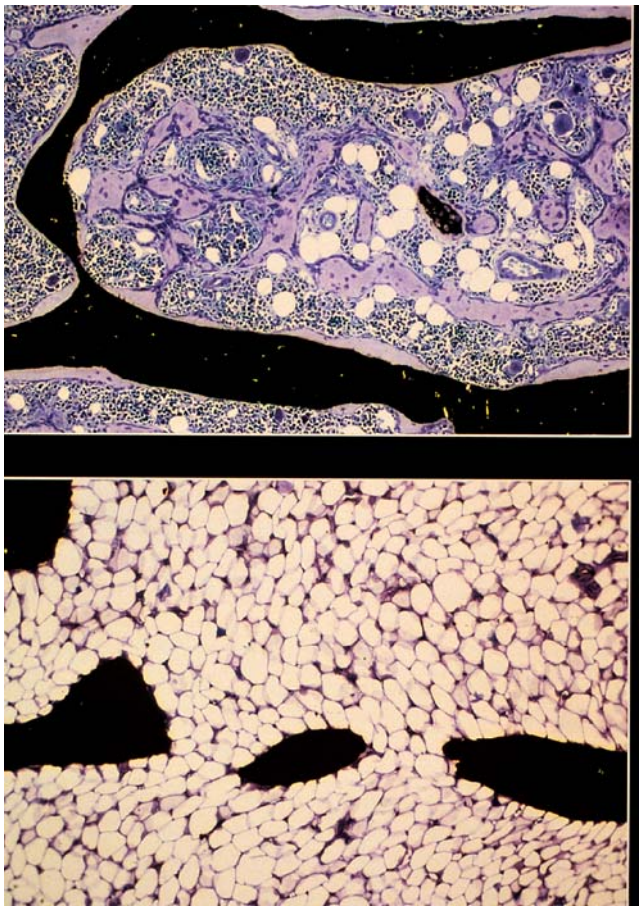
OSTEOCLAST SURFACE IS MARKEDLY DECREASED IN bFGF-TREATED OVX RATS

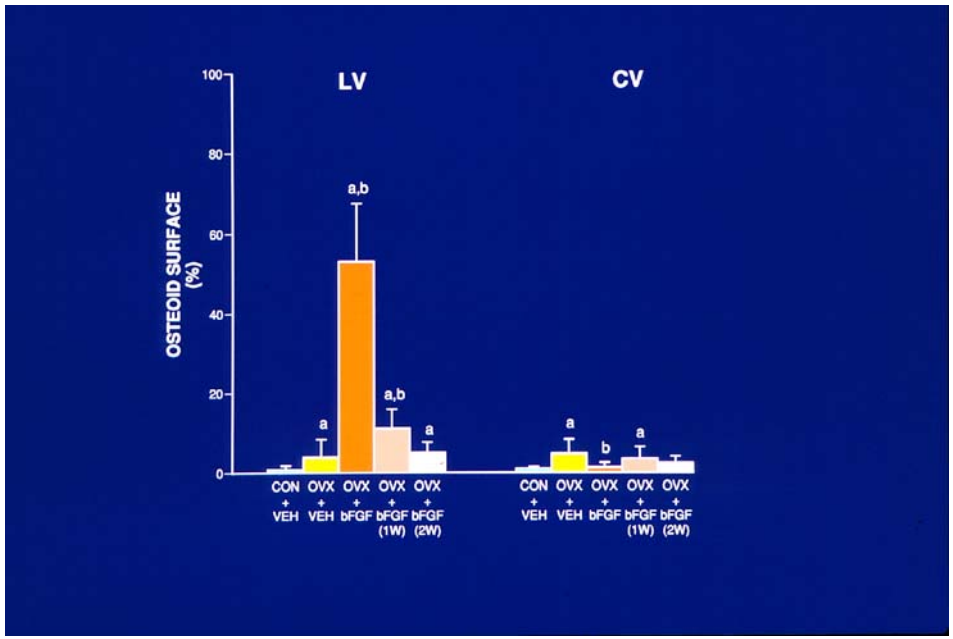


CONCLUSIONS (PILOT STUDY)

1. Basic FGF partially restores lost cancellous bone in osteopenic OVX rats after only 14 days of treatment.
2. Basic FGF markedly stimulates cancellous bone formation.
3. Basic FGF induces formation of osteoid spicules within bone marrow and osteoid bridges between bone spicules.
4. Osteoclast surface is markedly decreased by bFGF treatment.
5. Basic FGF impairs bone mineralization, but this process returns to normal soon after withdrawal of bFGF treatment.

bFGF DOES NOT HAVE A BONE ANABOLIC EFFECT AT SKELETAL SITES WITH FATTY MARROW





ADVERSE SIDE EFFECTS OF bFGF

Rats maintain normal body weights and remain outwardly healthy when treated with bFGF, BUT!

-Impaired bone mineralization

-Marked anemia

-Thickening of glomerular epithelium, which may result in impaired renal function

Therefore, rats cannot be treated long-term with bFGF!

EXPERIMENTAL OBJECTIVES

- To determine whether sequential treatment with bFGF and PTH can reverse severe cancellous osteopenia in the aged OVX rat.
- To determine whether prior systemic injection of mesenchymal stem cells (MSCs) enhances the osteogenic response to bFGF.
- To compare the bone anabolic effects of bFGF and an EP4 receptor agonist.

EP4 RECEPTOR AGONIST

- Prostaglandin E2 has a strong stimulatory effect on bone formation, but also adverse side effects.
- There are 4 cell surface receptors for PGE2 (EP1-4), and agonists specifically for the EP4 receptor appear to be bone selective.
- An EP4 agonist has been reported to induce formation of new bone spicules within bone marrow.

EXPERIMENTAL DESIGN

- Female Sprague Dawley rats were ovariectomized at 3 months of age and maintained untreated for the first year after surgery to allow for the development of severe cancellous osteopenia in the proximal tibia. Groups of baseline control and OVX rats that were 15 months of age were sacrificed at the end of this pretreatment period.

EXPERIMENTAL GROUPS

1. Baseline Control
2. Baseline OVX
3. OVX + MSC + bFGF*
4. OVX + bFGF*
5. OVX + EP4*

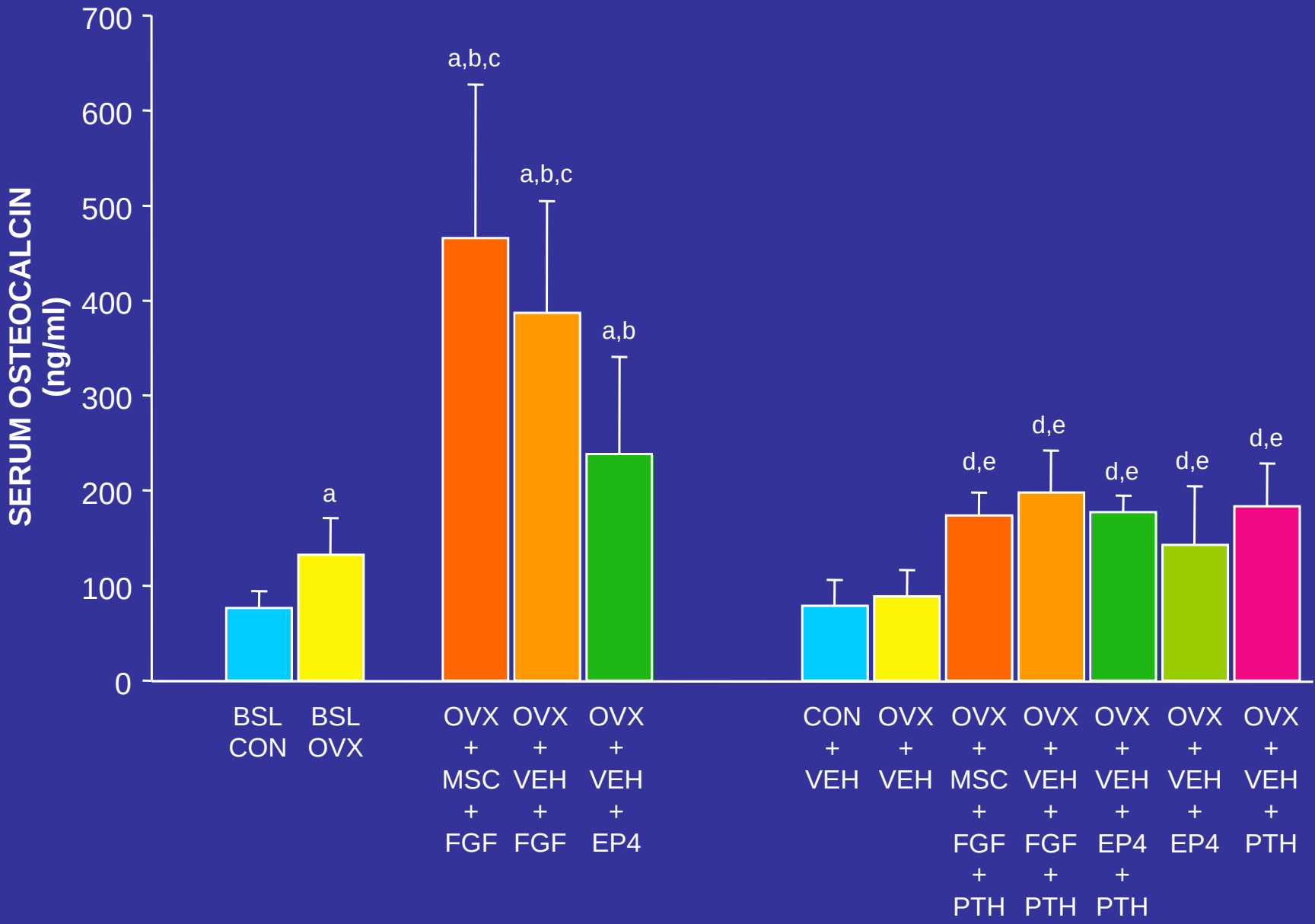
*Treatments with bFGF and EP4 consisted of daily SC injections for 3 weeks.

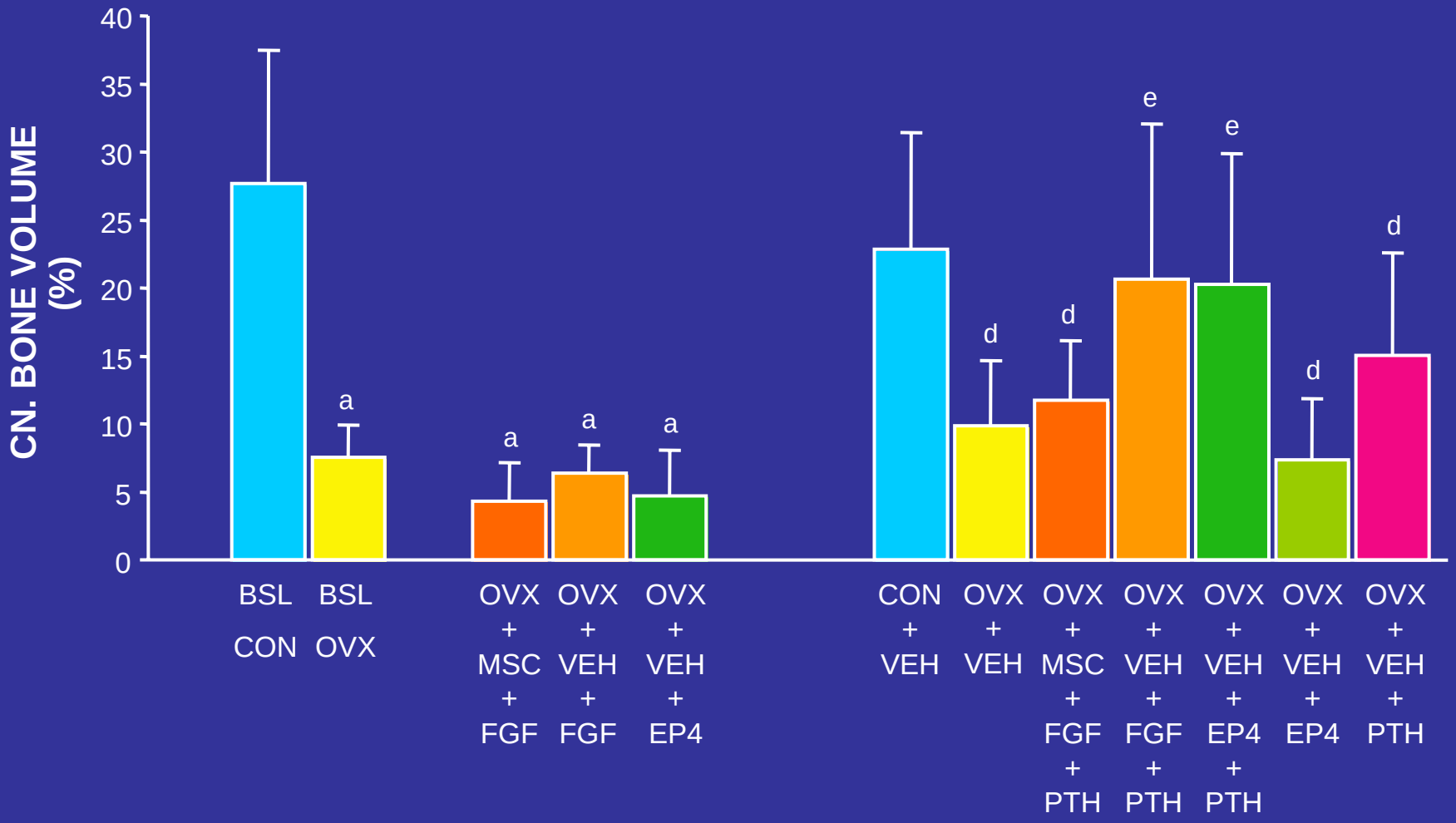
SYSTEMIC INJECTION OF MSCs

- MSCs were isolated from rat bone marrow and transfected with green fluorescent protein (GFP).
- GFP-labeled MSCs were injected in the right femoral artery of certain OVX rats at a dose of 2 million cells/kg body weight.
- A vasodilator (PTH) was injected SC at 0.5–1 hour prior to the intra-arterial injection of MSCs.
- OVX rats were sacrificed at 4h, 24h, and 7 days postinjection to detect MSCs in bone and various organs by fluorescence microscopy and immunohistochemistry.

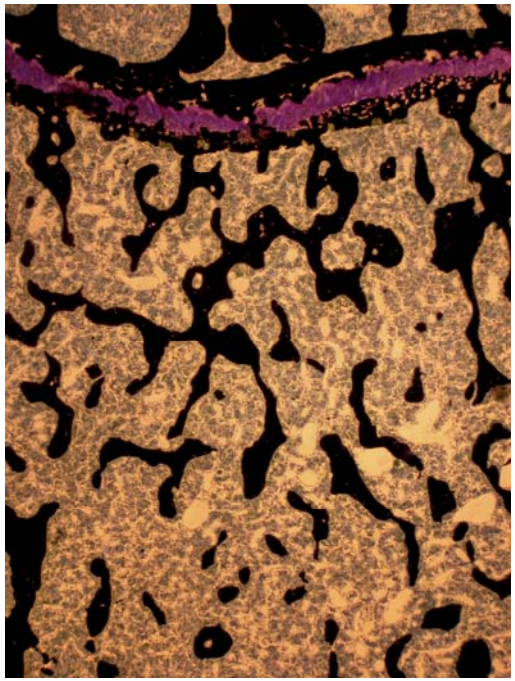
EXPERIMENTAL GROUPS

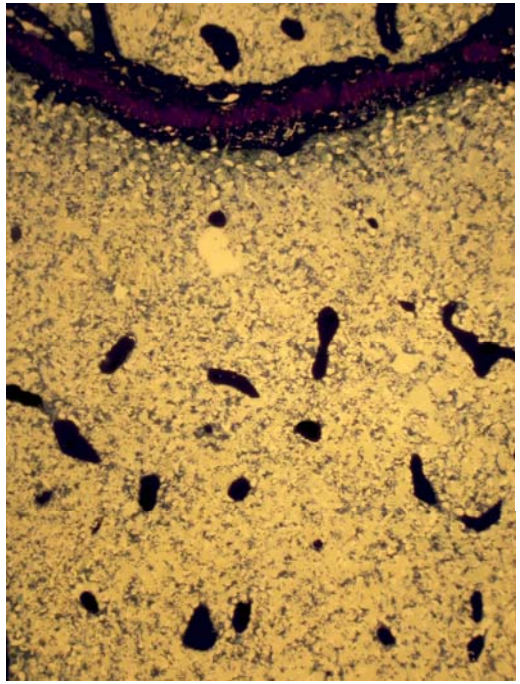
6. Control + Vehicle
7. OVX + Vehicle
8. OVX + MSC + bFGF (3 wks) + PTH (8 wks)
9. OVX + bFGF (3 wks) + PTH (8 wks)
10. OVX + EP4 (3 wks) + PTH (8 wks)
11. OVX + EP4 (11 wks)
12. OVX + PTH (11 wks)


SERUM OSTEOCALCIN (ng/ml)
700
600
500
400
300
200
100
0
a
a,b,c
a,b,c
a,b
d,e
d,e
d,e
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH


CN. BONE VOLUME (%)
40
35
30
25
20
15
10
5
0
a
a
a
a
d
d
d
e
e
d
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH

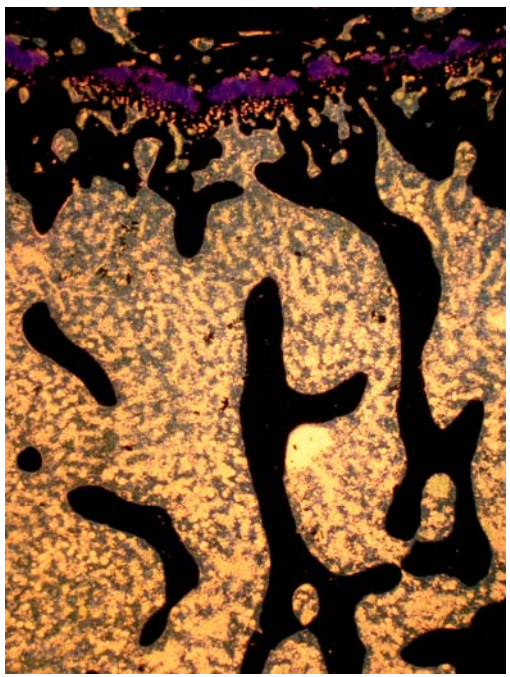
BSL
CTRL

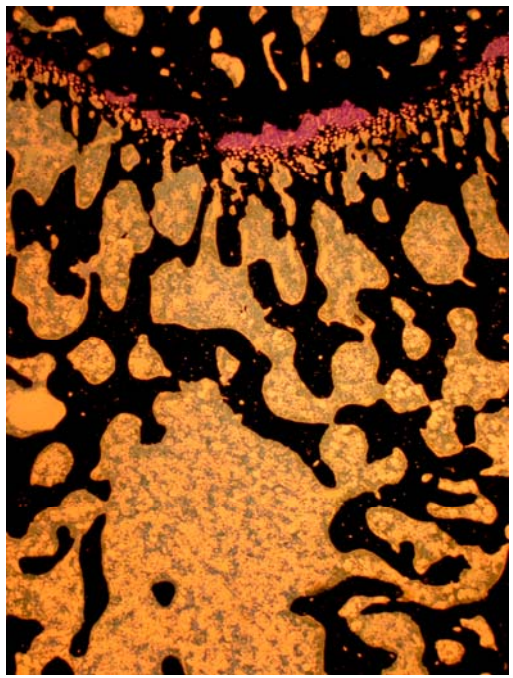




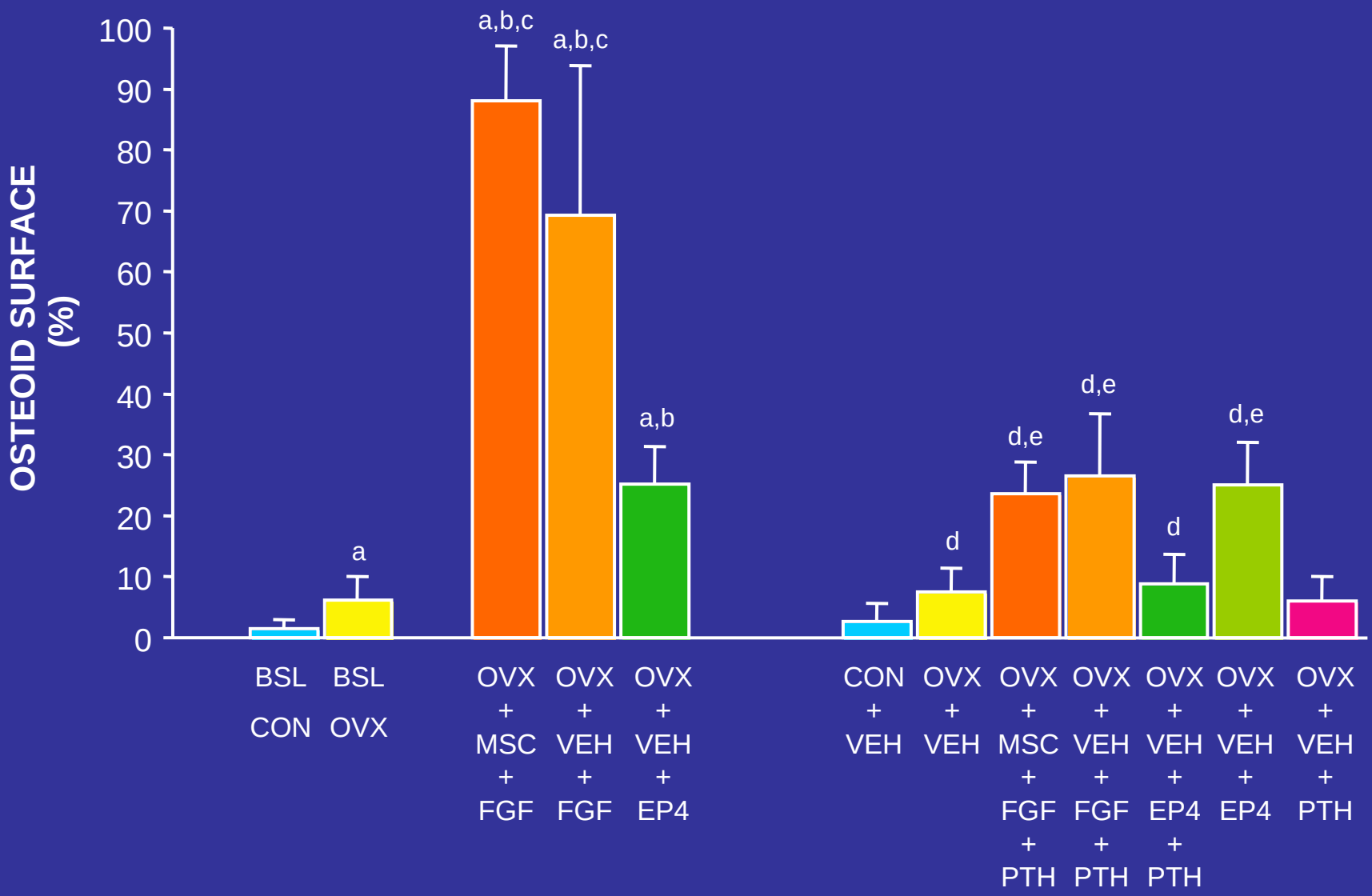
BSL
OVX

OVX
+
PTH

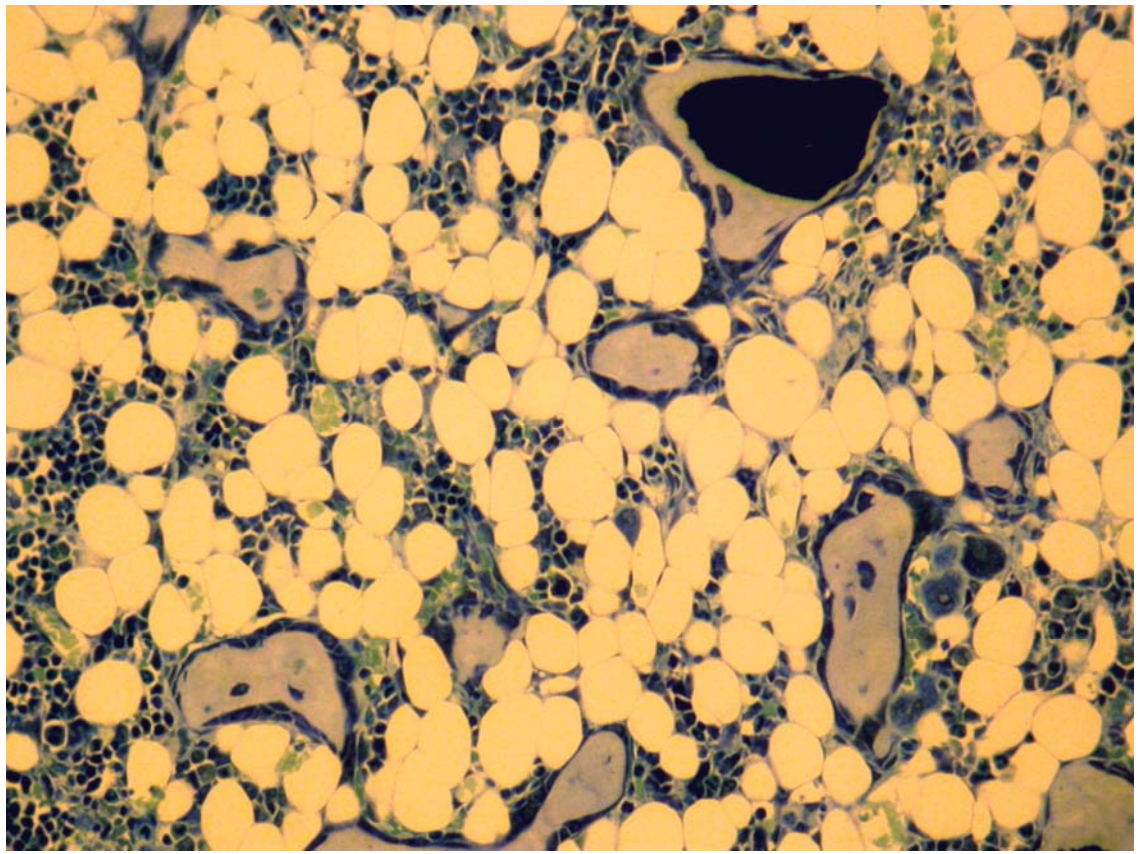


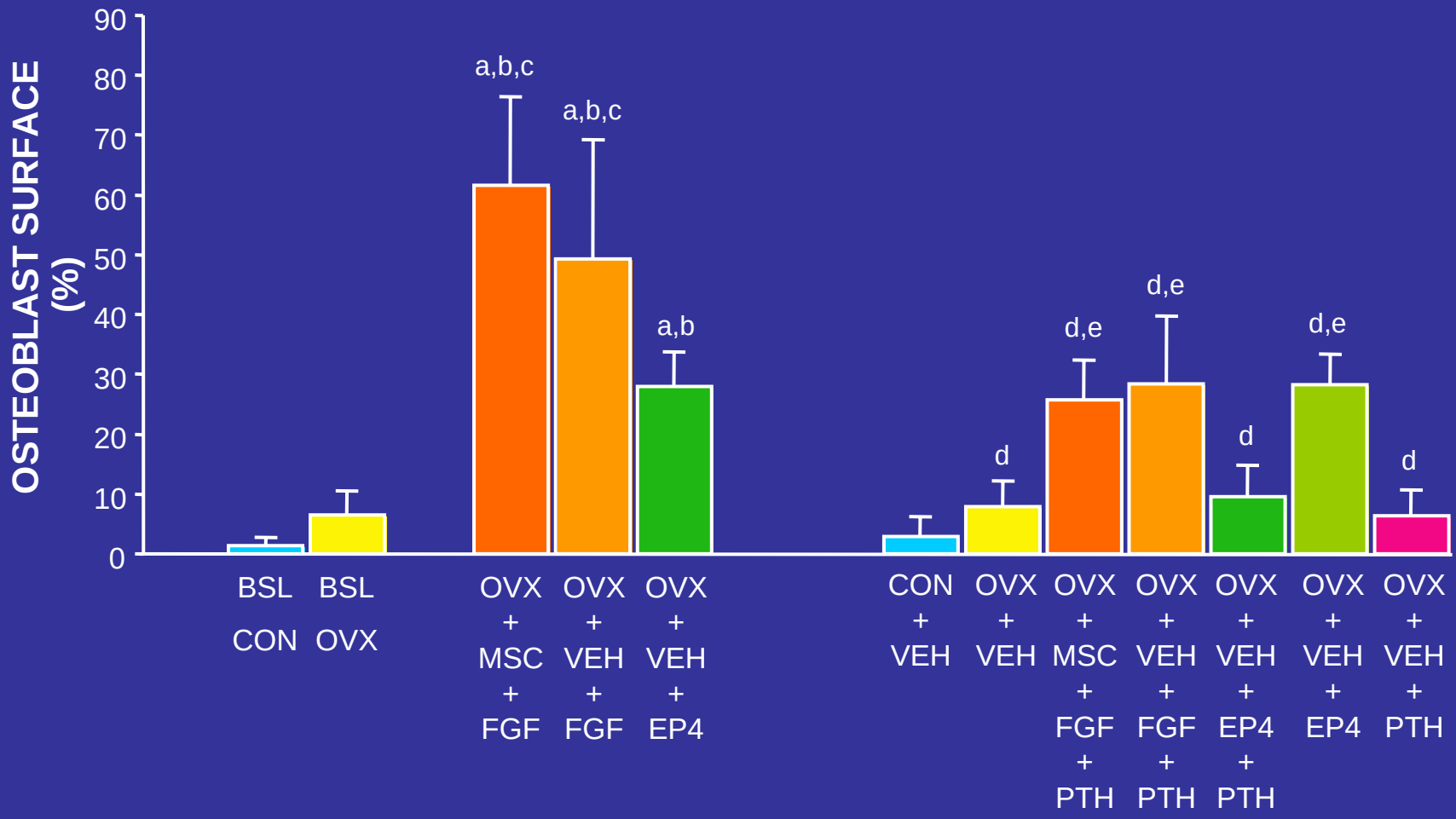


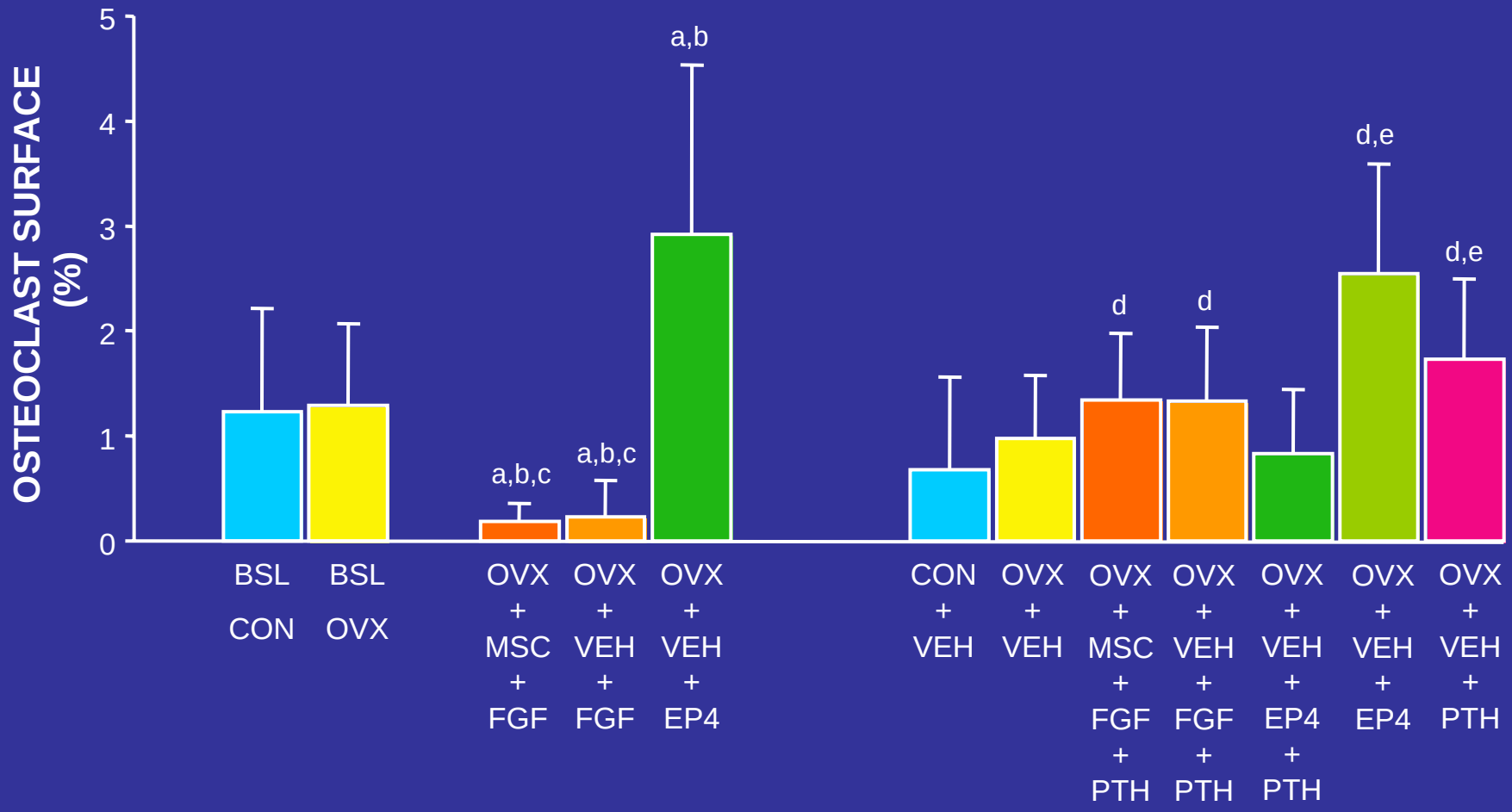
OVX
+
FGF
+
PTH

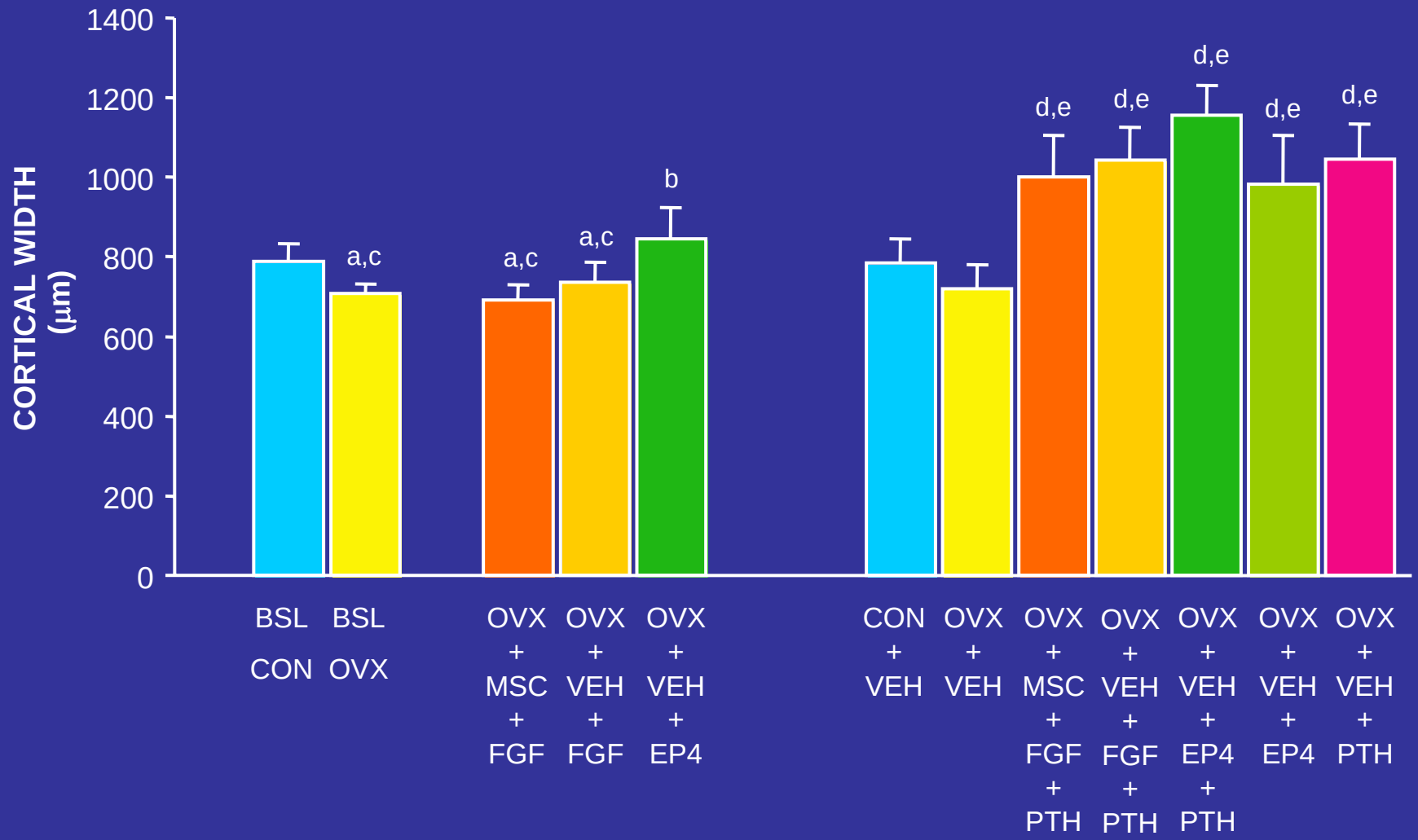

OSTEOID SURFACE (%)
100
90
80
70
60
50
40
30
20
10
0
a
a,b,c
a,b,c
a,b
d
d,e
d,e
d
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH

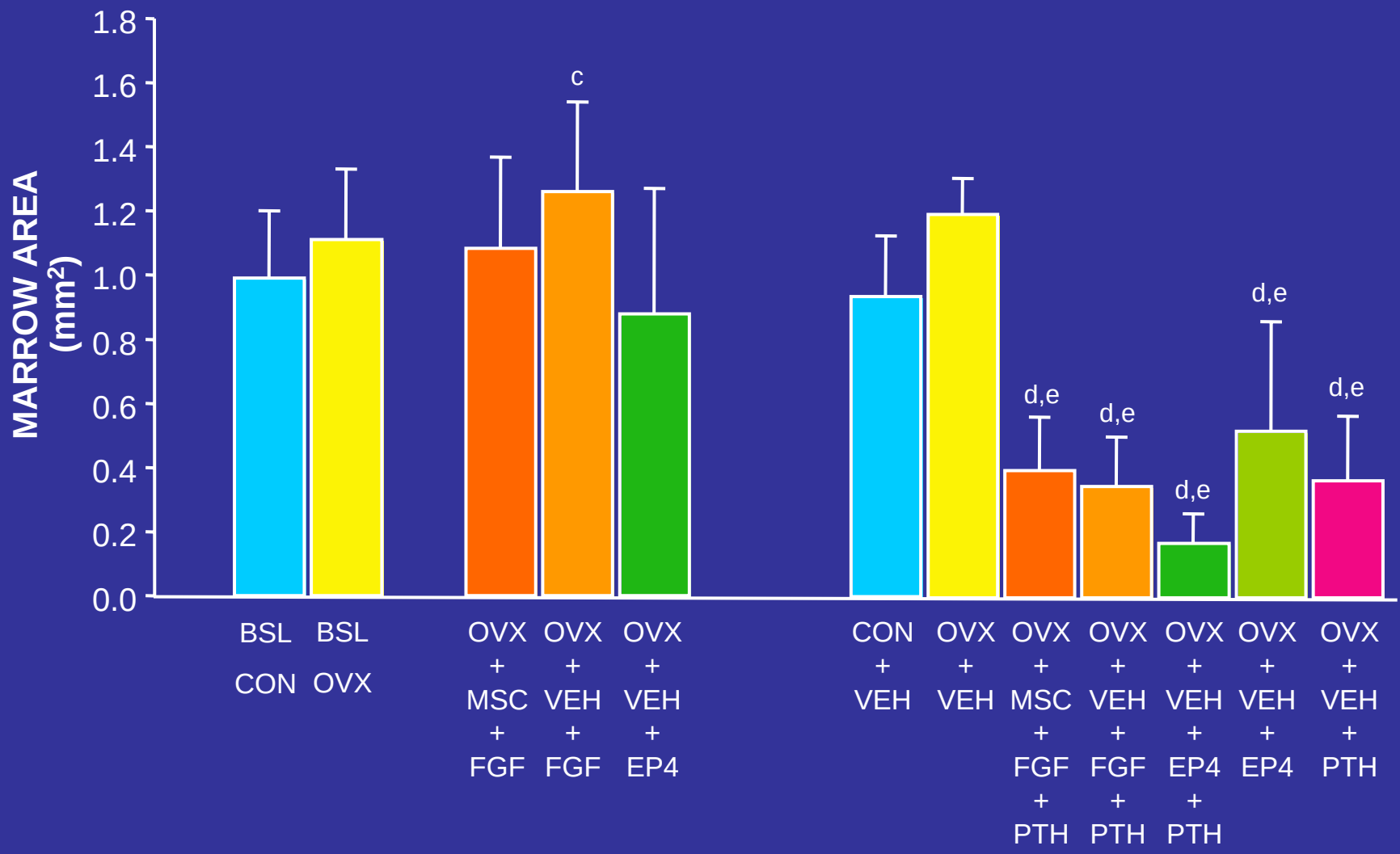
bFGF, BUT NOT EP4, INDUCED FORMATION OF OSTEOID ISLANDS WITHIN BONE MARROW

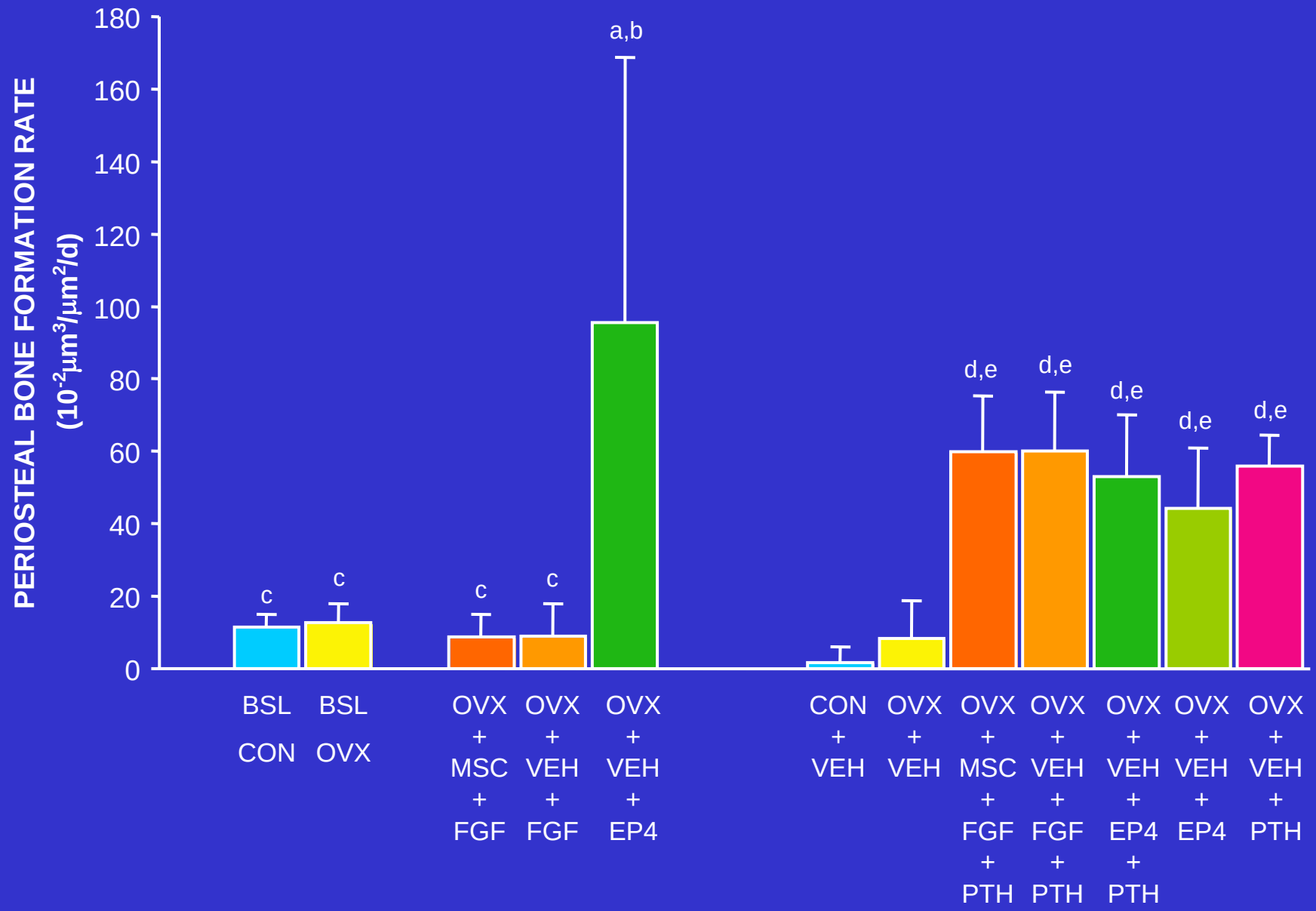


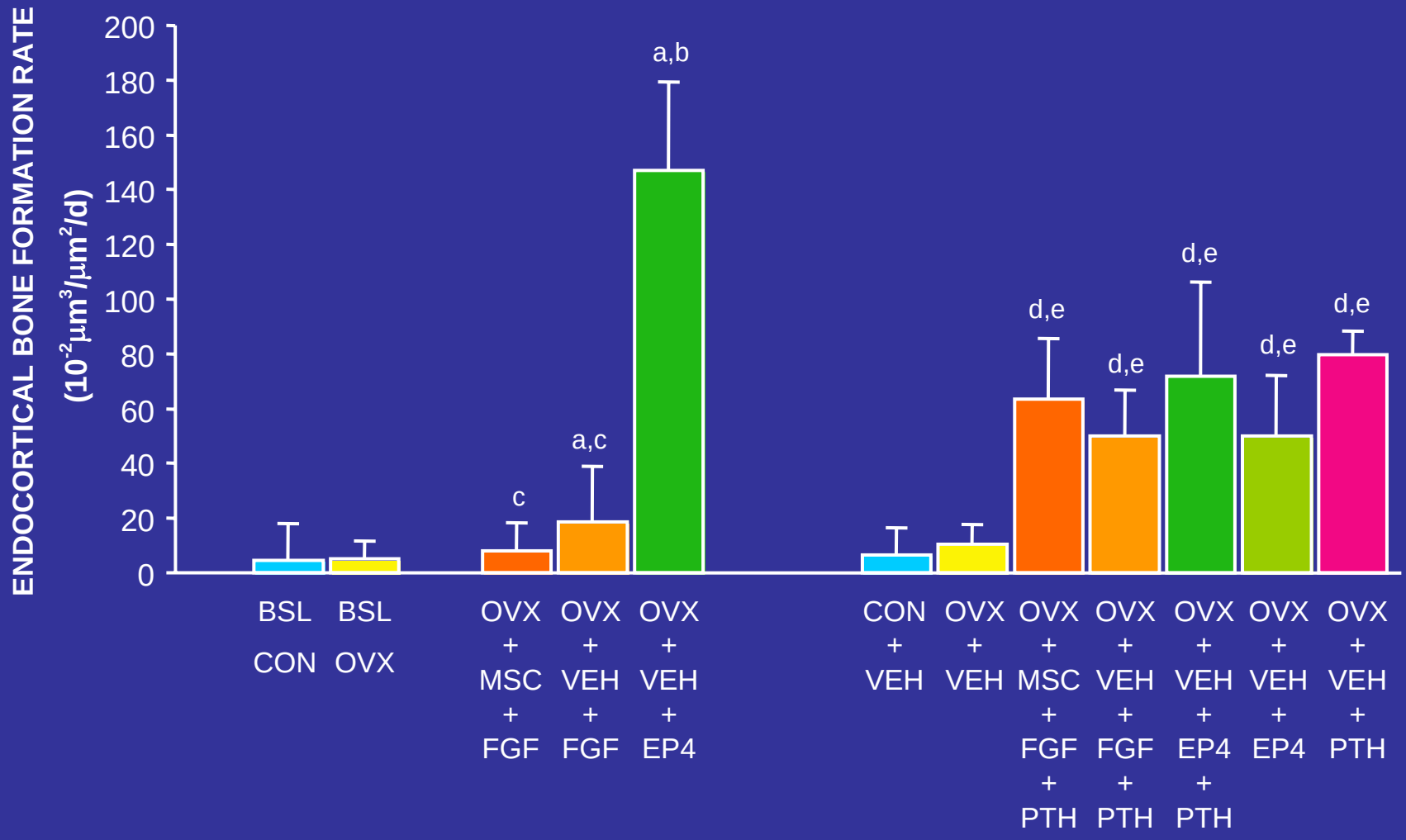

OSTEOBLAST SURFACE (%)
90
80
70
60
50
40
30
20
10
0
a,b,c
a,b,c
a,b
d
d,e
d,e
d
d,e
d
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH


OSTEOCLAST SURFACE (%)
5
4
3
2
1
0
a,b
a,b,c
a,b,c
d
d
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH

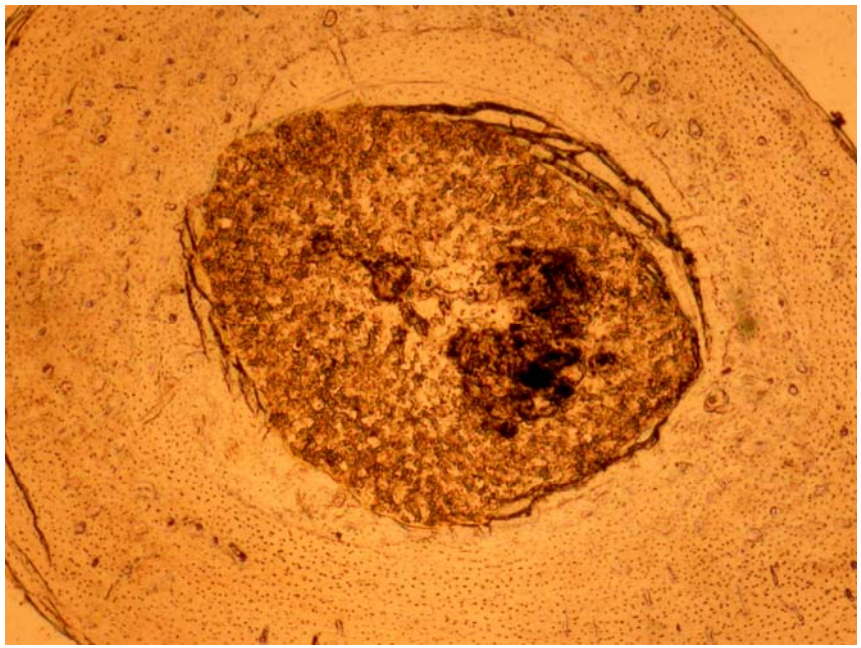

CORTICAL WIDTH (μm)
1400
1200
1000
800
600
400
200
0
a,c
a,c
a,c
b
d,e
d,e
d,e
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH


MARROW AREA (mm²)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
c
d,e
d,e
d,e
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH

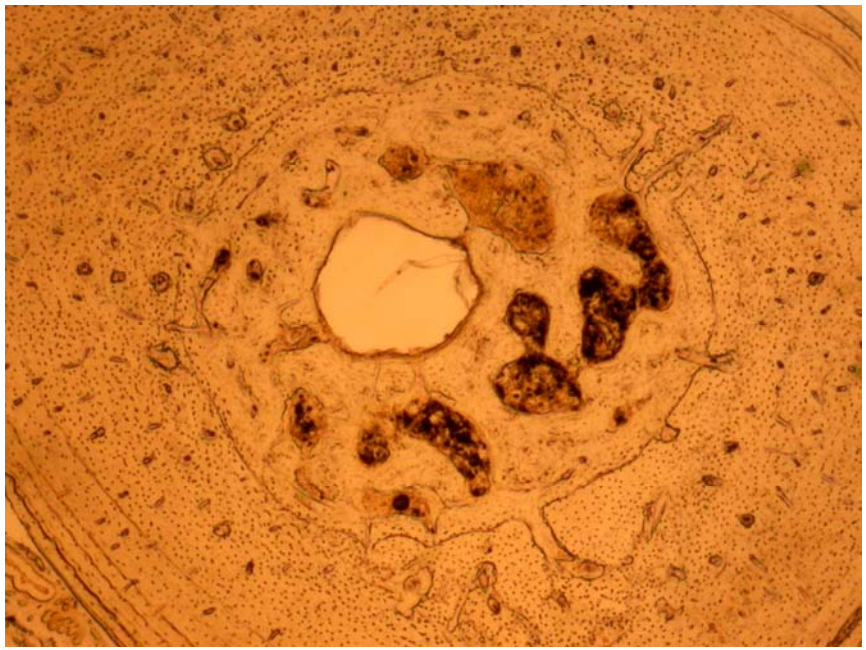

PERIOSTEAL BONE FORMATION RATE
(10-2µm3/µm2/d)
180
160
140
120
100
80
60
40
20
0
c
c
c
c
a,b
d,e
d,e
d,e
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH


ENDOCORTICAL BONE FORMATION RATE
(10-2µm3/µm2/d)
200
180
160
140
120
100
80
60
40
20
0
a,b
a,c
c
d,e
d,e
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH

EP4 TREATMENT MARKEDLY STIMULATES ENDOCORTICAL BONE FORMATION



BSL OVX



OVX + EP4

RESULTS OF REAL TIME-PCR ANALYSIS

bFGF

- Osteocalcin – 6.8*
- Type I collagen – 11.9*
- IGF-I – 1.8*
- BMP-2 – NC
- TGFβ-1 – NC
- VEGF – 2.2*
- COX-2 – NC
- OPG – NC
- RANKL – 5.5*
- Cathepsin K – 2.4*

EP4

- Osteocalcin – 4.3*
- Type I collagen – 3.6*
- IGF-I – 1.5*
- BMP-2 – NC
- TGFβ-1 – NC
- VEGF – 1.6*
- COX-2 – NC
- OPG – NC
- RANKL – 2.4*
- Cathepsin K – 2.2*

CONCLUSIONS

1. Basic FGF had the strongest stimulatory effect on cancellous bone formation in aged OVX rats and is the most promising bone anabolic agent for the reversal of severe cancellous osteopenia.
2. Prior intra-arterial injection of MSCs did not enhance the osteogenic effects of bFGF, BUT!!!
3. EP4 agonist stimulated cancellous bone turnover in aged OVX rats, but failed to restore lost cancellous bone mass.

CONCLUSIONS

4. Treatment of aged OVX rats with PTH alone tended to partially reverse severe cancellous osteopenia, whereas sequential treatment with bFGF + PTH and EP4 + PTH increased cancellous bone mass to the level of intact control rats.
5. Both the EP4 agonist and PTH increased cortical bone mass in the tibial diaphysis by stimulating both periosteal and endocortical bone formation.

FUTURE DIRECTIONS

- To advance bFGF as an osteoporosis therapy, the biological effects of the growth factor need to be bone selective!
- Identify ligands or small molecules that bind selectively to one of the cell surface receptors for bFGF.
- Use in vitro screening of these ligands followed by in vivo testing to determine if one of these ligands has the desired osteogenic effects with minimal side effects on non-skeletal tissues.

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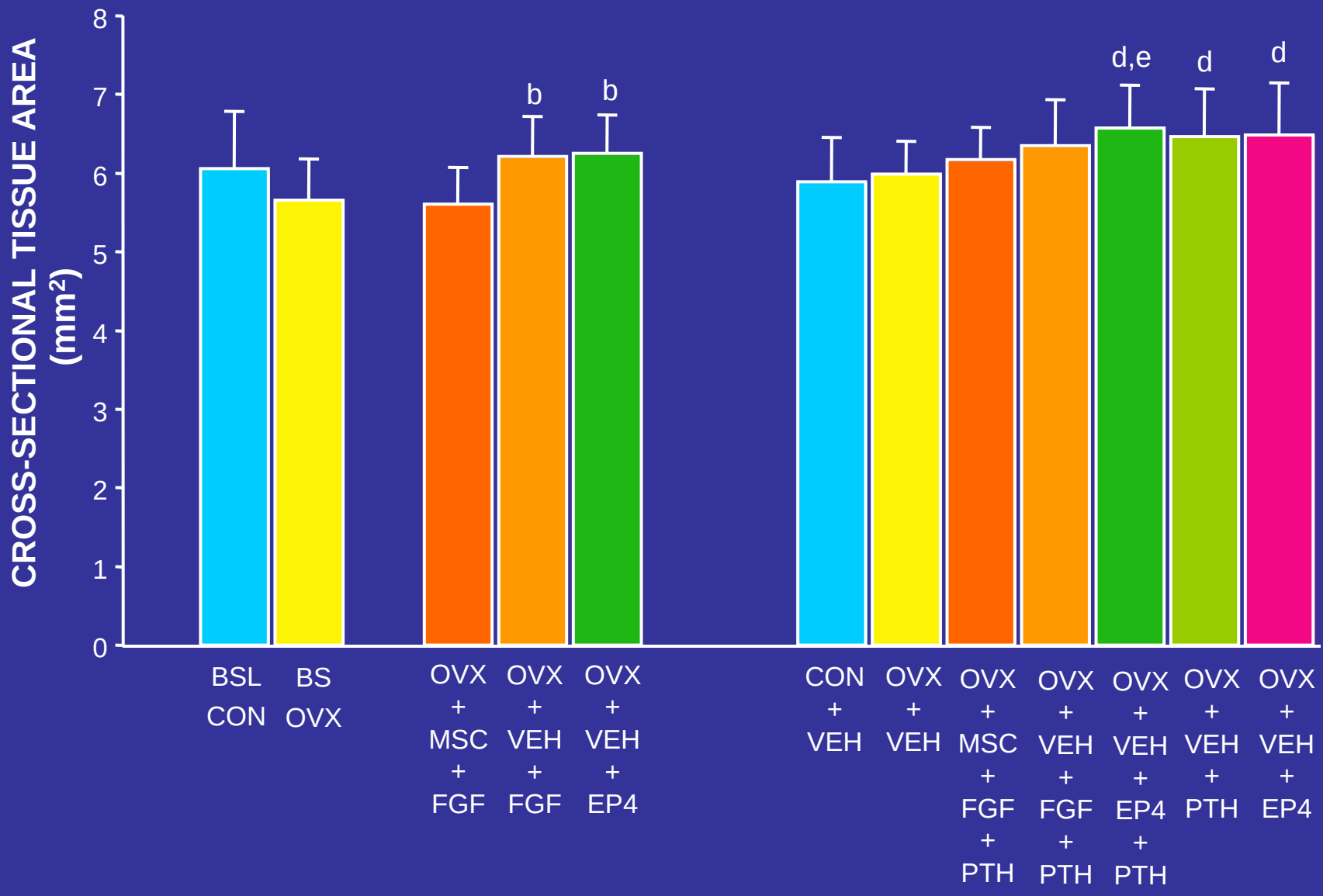
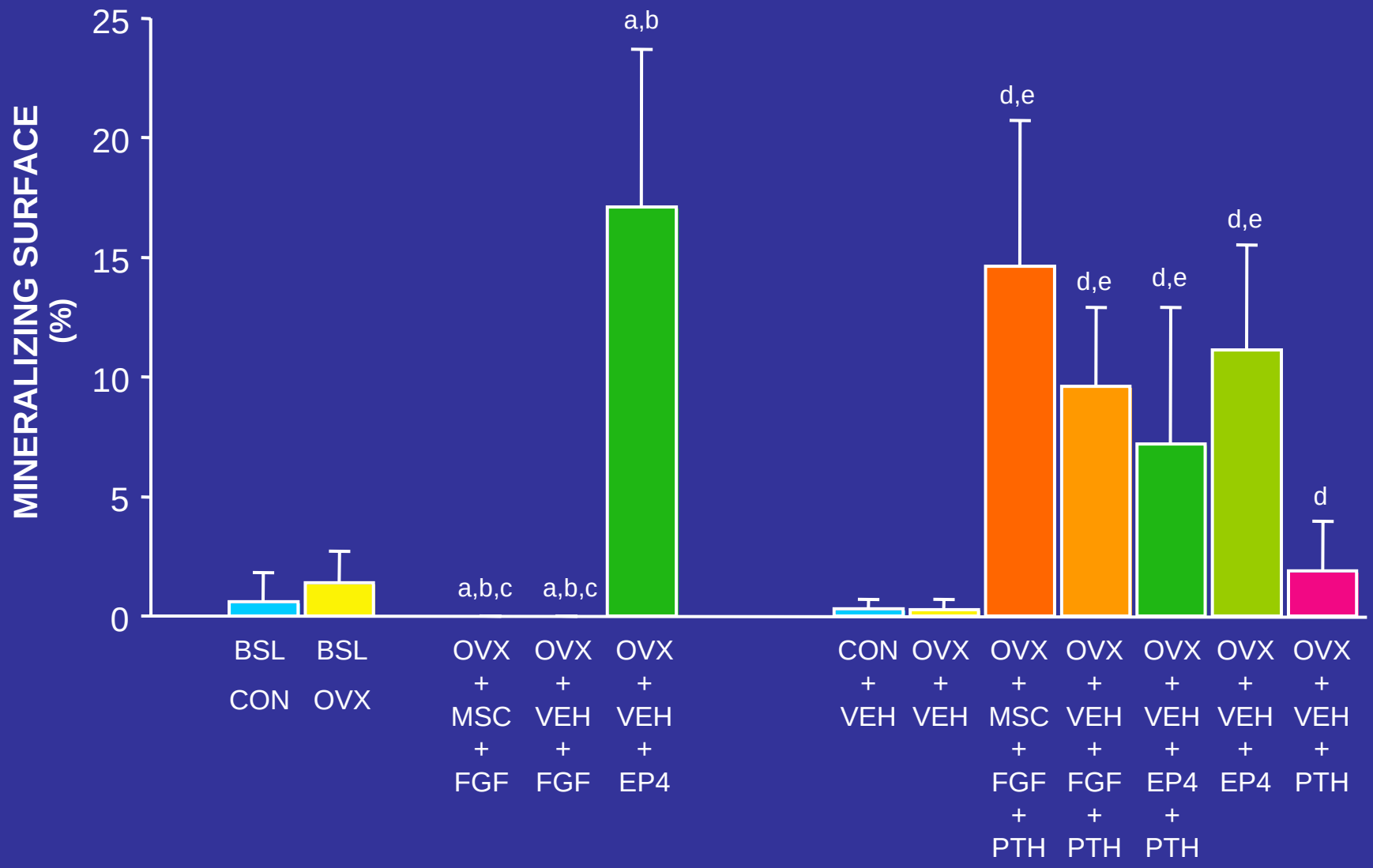
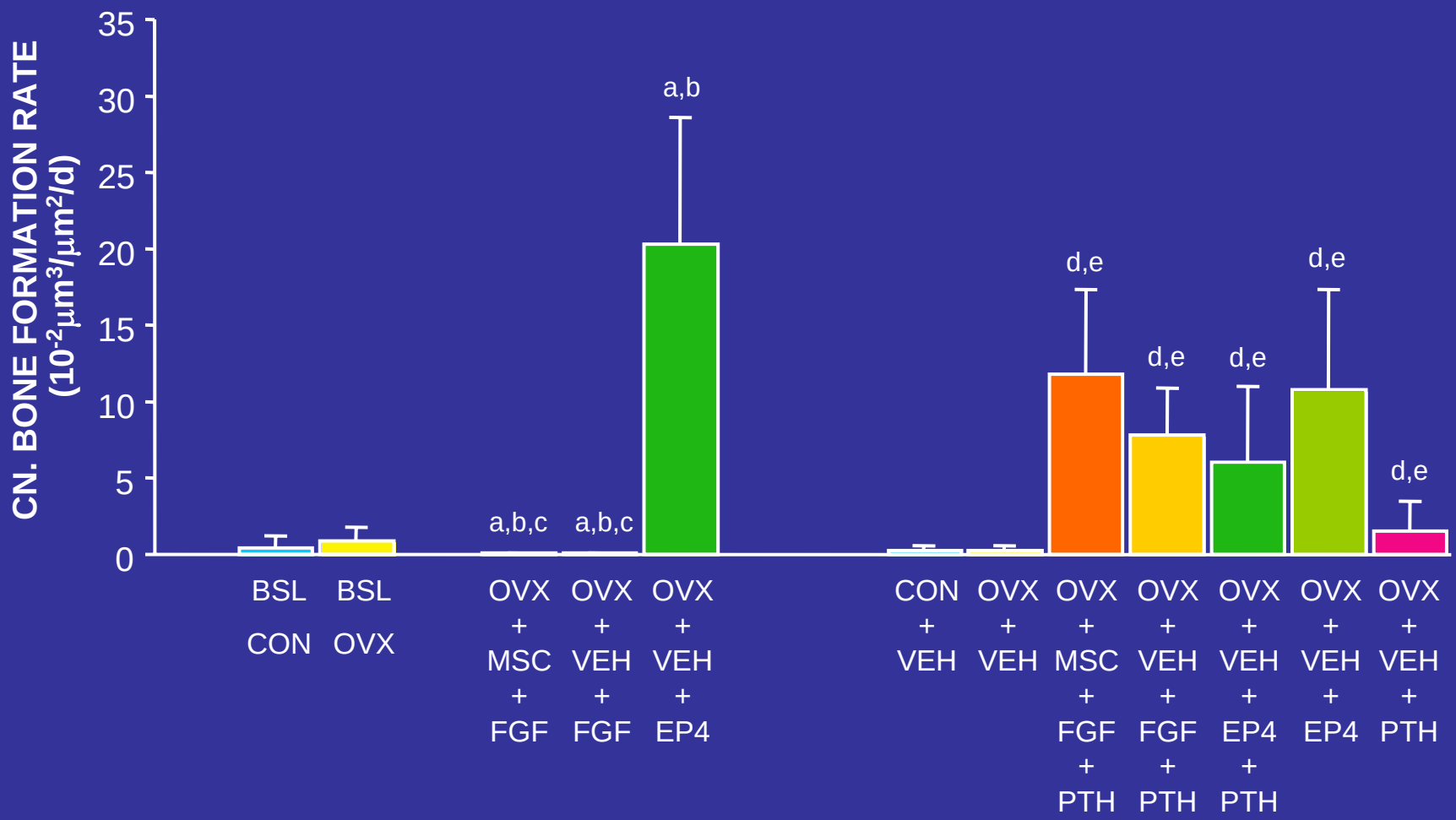
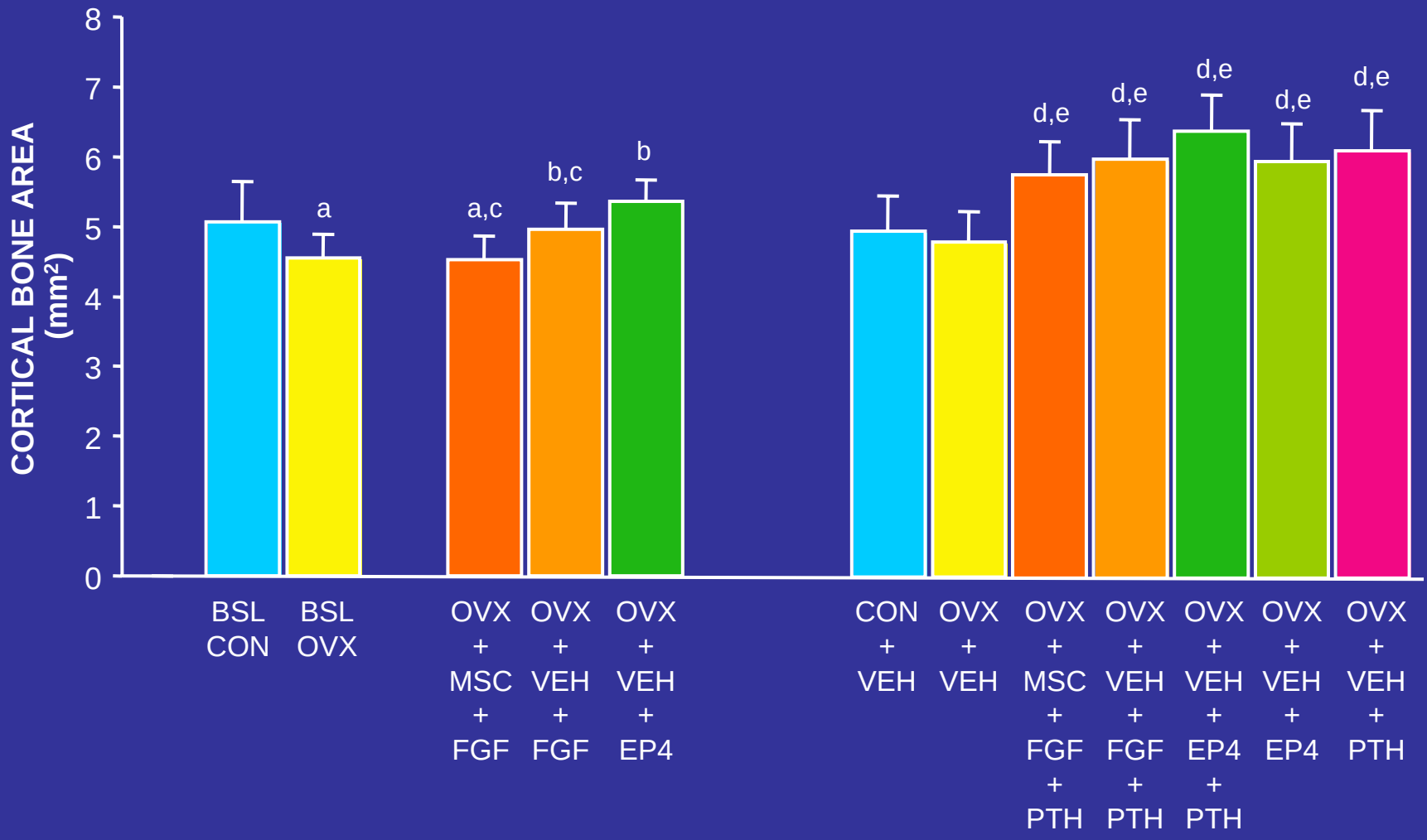

CROSS-SECTIONAL TISSUE AREA (mm²)
0
1
2
3
4
5
6
7
8
b
b
d,e
d
d
BSL CON
BS OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + PTH
OVX + VEH + EP4


MINERALIZING SURFACE (%)
25
20
15
10
5
0
a,b
a,b,c
a,b,c
d,e
d,e
d,e
d,e
d
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH


CN. BONE FORMATION RATE
(10-2µm3/µm2/d)
35
30
25
20
15
10
5
0
a,b
a,b,c
a,b,c
d,e
d,e
d,e
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH


CORTICAL BONE AREA (mm²)
0
1
2
3
4
5
6
7
8
a
a,c
b,c
b
d,e
d,e
d,e
d,e
d,e
BSL CON
BSL OVX
OVX + MSC + FGF
OVX + VEH + FGF
OVX + VEH + EP4
CON + VEH
OVX + VEH
OVX + MSC + FGF + PTH
OVX + VEH + FGF + PTH
OVX + VEH + EP4 + PTH
OVX + VEH + EP4
OVX + VEH + PTH